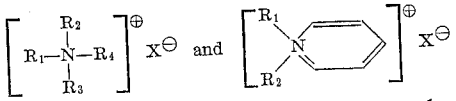
M, although generally the minimum amount of the salt will be about 0.2 M. In the preferred embodiments, wherein the pH is greater than about 4.0, the molarity of the salt solution is approximately 0.4–0.5 M. Of the foregoing salts, the alkaline earth salts, and especially calcium salts, are preferred.

Soluble cationic surfactants which may be used in the present invention are the art-recognized cationic surfactants which have the property of solubilizing soluble native collagen without enzyme treatment. These are, in general, quaternary ammonium compounds containing from 1 to 3 hydrogen or lower alkyl radicals (i.e. containing from 1 to 3 carbon atoms) and from 1 to 3 long-chain alkyl, aryl and/or arylalkyl hydrocarbons containing from 6 to 20 carbon atoms. The quaternary ammonium nucleus may be a part of a pyridinium group. The anion portion of the quaternary compound may be any appropirate water-solubilizing anion. Halides, such as chlorides and bromides, sulfates and methane sulfates are especially common.

The foregoing cationic surfactants are characterized by the general formulas:



each of R_1, R_2, R_3 and R_4 being selected from the group consisting of hydrogen, lower alkyl radicals containing from 1 to 3 carbon atoms in the alkyl group, and long-chain alkyl, aryl, and arylalkyl hydrocarbons containing from 6 to 20 carbon atoms, there being in the cationic surfactant from 1 to 3 hydrogen and lower alkyl radicals, and from 1 to 3 long-chain alkyl, aryl and arylalkyl radicals and X is a water-solubilizing anion. Typical quaternary ammonium compounds include, but are not limited to dodecyl dimethyl ammonium chloride, dodecyl dimethyl benzyl ammonium chloride, and dodecylamine hydrochloride. A concentration of cationic surfactant between about 0.01 M and 0.1 M should be used. The preferred concentration of cationic surfactant is between about 0.02 M and about 0.05 M.

Any proteolytic enzyme capable of digesting the telopeptide group may be used in the practice of this invention, such enzymes being referred to, for convenience, as the telopeptidases. This class of enzymes may be considered as composed of all proteolytic enzymes other than collagenase. Examples of enzymes that can be used include, but are not limited to, pepsin, trypsin, ficin, bromelin and papain. Still other proteolytic enzymes can be obtained by cultivating micro-organisms. For this purpose, *Bacillus subtillis, Streptomyces griseus, Streptomyces caespitocus, Aspergillus niger, Aspergillus saitoi, Aspergillus oryzae, Aspergillus niger van Tieghem, Trametes sanguinea* and *Paecilomyces varioti* are all useful, the foregoing being representative only of the micro-organisms which might be used. Still other enzymes are mentioned in the copending application of Ito and Kojima, Ser. No. 403,357 filed Oct. 12, 1964 and now abandoned.

As is well known in the art, the amount of enzyme required will vary widely depending upon the activity of the enzyme under the conditions of its use. It is contemplated, accordingly, that the amount of enzyme used will be sufficient to achieve the desired digestion. Smaller amounts of enzyme will, of course, lead to smaller digestion rates while higher amounts of enzyme will lead to faster digestion rates. In a normal, commercially practical process, the amount of enzyme required will be between about 0.01% and 5.0% based on the weight of the dry insoluble collagen initially used.

The enzymatic reaction may be carried out at any convenient temperature, but must be carried out at a temperature below the denaturation temperature of the collagen. Accordingly, temperatures in excess of about 35° C. should be avoided. Since the presence of the salt may effect the denaturation temperature, however, there may be cases where the permissable maximum temperature is even lower. Usually commercially useful enzyme activity is not obtained at temperatures below about 0° C.

The concentration of the collagen fibers before enzymatic digestion may be at any convenient level. It is preferred that the concentration of insolbule collagen in the salt solution be no more than about 100 grams/liter. Under these conditions, the enzyme treatment and the extraction of the solubilized collagen will occur substantially simultaneously.

The present invention is further illustrated by the following examples.

Example 1

The butt portion of a freshly slaughtered steer hide or steer hide trimmings which had been salted was washed with water after removal of hair. The grain layer and flesh side of the steer butt or trimmings were removed, and the portion rich in collagen was taken out as a raw material. The raw material thus obtained was washed again with water, immersed in a 10% aqueous saline solution and finally disintegrated into small pieces at a low temperature by the use of a mincer. The minced material was washed with 10% aqueous saline solution to remove proteins such as albumin, globulin and the like, and then fully washed with a 0.15 M citric acid solution to dissolve out any small amount of acid-soluble collagen contained in the material. The resulting material was thoroughly washed with water, dehydrated with alcohol, defatted with alcohol-ether (1:1) and dried. The dried material thus obtained was the so-called insoluble collagen. The insoluble collagen was used as the raw material in Examples 2 to 14 below.

Example 2

To 600 mg. of the insoluble collagen prepared in Example 1 was added 100 ml. of 0.5 M calcium chloride solution which had been adjusted to a pH of 6.2 with acetic acid. After the resulting mixture was left alone for about one hour, the enzyme produced from the *Streptomyces griseus* described in U.S. Patent No. 3,127,327 (available under the trademark Pronase-P) was added in an amount of 2% by weight of the dry collagen, and the mixture was subjected to moderate agitation at a temperature of 20° C. After two days of agitation, the mixture turned into a completely clear solution having a high viscosity. The clear solution was then filtered through a glass filter to remove the small amount of non-collagenous material remaining in the solution.

The filtrate thus obtained was dialyzed against the same buffer solution as that used in the solubilization of the collagen, with the result that the non-collagenous fraction decomposed by the enzyme was removed in an amount of from 5 to 10% by weight of the starting collagen. The tetrasodium salt of ethylenediamine tetraacetic acid was added to the collagen solution remaining after the dialysis in an amount equimolar to the calcium ion present. When the pH of said solution was adjusted to 7 to 8 with sodium phosphate, the collagen fiber was reconstituted with a yield of 100%. (In referring to recovery of 100% of the collagen here and in the following examples, reference is made to recovery of 100% of the portion of the collagen molecule having the characteristic collagen structure. In all cases there is a weight loss between the initial raw collagen and the recovered fibers of 5% to 10%, which is believed to represent the telopeptide end linkages which are digested.)

The same reconstitution could also be achieved if the filtrate of the solubilized collagen is dialyzsed or extruded through spinnerettes against water or 1% NaCl.

Example 3

To 6 grams of the insoluble collagen of steer hide prepared by the procedure described in Example 1 having a water content of about 18% by weight was added